

A Study of *Sarcocystis* Infection in Mincemeat Using Digestion Method in Ghazvin, Iran

Jaber Davoudi^a, Afshin Bahman Shabestari^a, Peyman Mozahheb^a, Roghayeh Norouzi^b,
Mohammadreza Maadina^a, Razzagh Mahmoudi^{c*}

^aDepartment of Veterinary Parasitology, Islamic Azad University, Abhar-Branch, Abhar, Iran.

^bDepartment of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.

^cMedical Microbiology Research Center, Qazvin University of Medical Sciences, Qazvin, Iran.

*Correspondence should be addressed to Dr. Razzagh Mahmoudi, Email: r.mahmodi@yahoo.com

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Background & Aims of the Study: Sarcocystosis is a zoonosis appeared in domestic animals caused by various species of Sarcocystis. This protozoan disease has worldwide distribution among human and many species of animals. Humans acquire infection by eating of raw and under cooked beef, pork or mincemeat containing schizonts of *Sarcocystis hominis* and *S. suihominis*. The aim of present study is to detect prevalence of the *Sarcocystis* spp. infection in mincemeat samples at Ghazvin province of Iran.

Materials & Methods: Three hundred mincemeat samples of 150 sheep and 150 cattle were collected from butchers (in spring 2013) in different areas of Ghazvin province, Iran. The statistical analysis was done by independence sample t test, using SPSS ver. 22.0.0 (Chicago, IL, USA).

Results: the finding of this study showed that the highest prevalence of Sarcocystis infection rate was observed in cattle (92.8%) and the lowest of that was evident in sheep (85.6%). The highest infection rates in both types of minced meat samples were in May (45 and 49 minced meat of sheep and cattle, respectively).

Conclusions: The results revealed that Ghazvin province has the highest Sarcocystis infection rate. Regarding to the high prevalence of Sarcocystis contamination in this study, prevention of eating raw or under-cooked meat is strongly recommended.

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Background

Sarcocystis is an obligatory intracellular protozoan parasite effecting humans and animals. Many researchers from different areas of the world reported the distribution of the parasite worldwide. Life cycle of the parasite includes an intermediated host (man or herbivores animals) and definitive host (man and carnivores animals). Carnivorous such as canine and feline family, infect environment via faeces by passing 200 million oocyst during infection period (1-2).

Human's infection caused by consumption raw and under cooked beef, pork or mincemeat containing schizonts of *Sarcocystis hominis* and *S. suihominis*. The prevalence of sarcocystosis has been investigated in slaughtered food animals in different parts of the world by different researchers such as Ginawi et al., 1997; Pena et al., 2001; Savini et al, 1992 and Beyazit et al., 2007 (3-6); also, in Iran by Valinezhad et al., 2008; Daryani et al., 2006; Atashparvar et al., 2001; Razavi et al., 2003 and Arshad et al., 2007, different methods have been used which indicating the infection of 3.5% to 100% (7-11). Intestinal sarcocystosis

infection in human had some clinical signs such as disturbances of digestive system such as nausea, vomiting and diarrhea (12) especially in immune-compromised patients (13).

Li et al (2006) in the study of human experimental infection model reported that some clinical signs appear after 5 hours such as abdominal distension, watery diarrhea, vomiting, chilling and fever, dizziness, headache, joint and muscle ache, epigastralgia and anorexia (14). The Un-sporozized sporocysts and sporocysts forms were found in the faeces 10 days after infection and 12th day, respectively. Muscular sarcocystosis in human beings are created by *S. lindemanni*. The infection is induced by ingestion of oocysts which passed through faeces of infected dogs (15).

Aims of the study:

The main objective of this study was to determine the prevalence of Sarcocystis infection of mincemeat from cattle and sheep in Ghazvin province, Iran.

Materials & Methods

Sampling preparation

In this cross-sectional study, three hundred mincemeat samples of 150 sheep and 150 cattle were collected from butchers (A total of 50 samples were collected every month in spring 2013) in different areas of Ghazvin province, Iran. The digestion method was used for detection of Sarcocystis bradyzoites (14-15).

Detection of Sarcocystis

Approximately 50 g of mincemeat were digested for 30 min at 40°C in 50ml of digestion medium containing pepsin, HCl and NaCl in 500 ml of distilled water. Then, the digestate was poured through a fine-meshed sieve into a beaker and the filtrate was allowed to settle for 30 min. After separation of supernatant fluid, the sediment was stained by Giemsa and examined microscopically for detecting Sarcocystis (16).

Statistical Analysis

Significant ($P < 0.05$) between cattle and sheep mincemeat samples was analyzed by independence sample t test, using SPSS ver. 22.0.0 (Chicago, IL, USA).

Results

The prevalence of microscopic sarcocysts in mincemeat samples of sheep and cattle in Ghazvin, Iran, is shown in Table 1. According to Statistical analysis and independence sample t test, no statistically significant differences between the mincemeat samples of in cattle was observed during the months of sampling. In the case of sheep minced meat highest percentage of contamination in Ma (41%) and the lowest was in April (49%). However, the highest prevalence of Sarcocystis infection rate was observed in cattle (92.8%) and the lowest of that was evident in sheep (85.6%).

Table 1) Sarcocystis infection in mincemeat samples of cattle and sheep in Gazvin province, Iran

Type of mincemeat (n)	No.Positive (%)			Total no.Positive (%)
	1 st month	2 nd month	3 rd month	
Mincemeat of Sheep (150)	35	45	44	124 (85.6) ^a
Mincemeat of Cattle (150)	41	49	48	134(92.8) ^a

n: Number of samples

^a According to independence sample t test significant difference does not exist between sheep and cattle samples ($P > 0.05$)

Discussion

Conventional methods for detection of Sarcocystis in food samples including digestion methods are microscopic (11). Our study showed that the digestion method is appropriate to detect infected samples. Similar studies also confirmed the desirability of digestion method compared with microscopic examination. It is suggested that digestion techniques would be a profitable method to implement in investigation of Sarcocystis researches. They were unable to find cysts by microscopic examination while 98% were positive by digestion method (11).

In many studies at different parts of the world including Iran (7-12), the prevalence of sarcocystosis in slaughtered animals has been investigated, the results showed that contamination rate was 3.5% to 100%. This study indicated that mincemeat of sheep and cattle were infected with Sarcocystis in considerable percentage in 85.6% and 92.8%, respectively, thus, high contamination of environment with different strains of this parasite is possible. There is little investigation on mincemeat contaminated with Sarcocystis in Iran.

Hamburger is one of the most pleasant fast foods in many countries all over the world. Iranian traditional hamburger is mainly composed of minced meat (cattle, sheep, goat, camel or buffalo compose), onion, garlic, wheat flour, vegetable protein, and oils, some spices (pepper, salt and turmeric). Human's infection caused by consumption raw and under cooked beef, pork or mincemeat containing schizonts of Sarcocystis (2,13,17). Sarcocystis infection in hamburger samples in Yazd market, the results showed that 77.9% of all tested hamburger samples were infected with Sarcocystis spp. The infection rate in the traditional hamburger (87%) was significantly ($p < 0.05$) higher than the industrial ones (67.8%) (17). Rahdar and Salehi (2011), in a similar study, indicated an infection rate of 56.0% for Sarcocystosis in hamburgers in

Ahvaz, Iran (15). Recently, Nematollahi et al. in the year 2013 showed that the prevalence of Sarcocystis spp. in both traditional and industrial hamburgers was 56.25% in Tabriz, Iran. Their study performed by both impression smear and peptic digestion methods. This issue must be highlighted that the infection rate is not only attributed to the geographical area or age and gender of the intermediate host but also crucially influenced by the method applied to detect Sarcocystis infection (18). While, both the impression smear and peptic digestion methods showed infection rates of 47.9 to 56.0% at various parts of Iran, Jahed-Khaniki and Kia (2006) reported an infection rate of 6.25%, using histological method, in Garmsar, Iran (18).

Prayson et al (2008) found Sarcocystis spp. in two out of eight examined hamburger brands in USA, using histological method (13). The results of Pena et al (2001) on raw beef prepared for kibbe in 25 Arabian restaurants in Sao Paulo, Brazil, showed that all samples contained sarcocysts (4). Reported prevalence of sarcocystosis in other areas include: Saudi Arabia (camels: 88.35%) (19), western Australia (cattle: 52%) (20) and Sri Lanka (cattle: 69.3%) (21).

Several researchers reported that there are considerable infection rate in cattle of Australia (52%), Brazil (75%), Germany (63%) and New Zealand (92%) (22,23).

Conclusion

The results of this study revealed that Ghazvin province has the highest Sarcocystis infection rate. Regarding to the high prevalence of Sarcocystis contamination in this study, prevention of eating raw or under-cooked meat is strongly recommended. To eliminate the infection in meat products can be used freezing (Keeping at -20°C for 1 day or -4°C for 2 days) and heating (temperature 70°C at the center of product). Finally, it must be highlighted that to recline the effects of the infection, meat and

related products should be kept at frozen for at least 3-5 days before consumption.

Footnotes

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Conflict of Interest:

The authors declared no conflict of interest.

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